JOURNAL OF CLINICAL MICROBIOLOGY, June 2005, p. 2651–2655 Vol. 43, No. 6
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.6.2651–2655.2005

Novel Chemically Modified Liquid Medium That Will Support the Growth of Seven *Bartonella* Species

Ricardo G. Maggi, Ashlee W. Duncan, and Edward B. Breitschwerdt*

Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606

Received 29 October 2004/Returned for modification 24 December 2004/Accepted 30 January 2005

Bacteria of the genus *Bartonella*, a member of the *Alphaproteobacteria*, are fastidious, gram-negative, aerobic bacilli that comprise numerous species, subspecies, and subtypes. In human and veterinary medicine, species isolation remains a vital component of the diagnostic and therapeutic management of *Bartonella* infection. We describe a novel, chemically modified, insect-based liquid culture medium that supports the growth of at least seven *Bartonella* species. This medium will also support cocultures consisting of different *Bartonella* species, and it facilitated the primary isolation of *Bartonella henselae* from blood and aqueous fluid of naturally infected cats. This liquid growth medium may provide an advantage over conventional direct blood agar plating for the diagnostic confirmation of bartonellosis.

Due to their zoonotic potential, their vector transmission, which includes sandflies, lice, fleas, and ticks, and their frequent adaptation to a mammalian reservoir host, *Bartonella* species are considered among the newest and most significant emerging pathogens (1, 3, 8, 12, 27, 29, 39, 45). These bacteria are highly adapted to a mammalian reservoir host; further, these organisms have been shown to cause a long-lasting intraerythrocytic bacteremia in both humans and animals (11, 14, 22, 24, 25, 37). *Bartonella* species are also the causative agents of Carrion's disease (Oroya fever and verruga peruana) (*Bartonella bacilliformis*) (6), trench fever (*B. quintana*) (11, 40, 43), endocarditis (*B. elizabethae*, *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, *B. washoensis*, *B. clarridgeae*) (3, 7, 9, 16, 17, 21, 40, 44), bacillary angiomatosis in immunocompromised patients (*B. quintana*, *B. henselae*) (14, 30, 48), neuroretinitis (*B. grahamii*) (28), and cat scratch disease (*B. henselae*, *B. clarridgeiae*) (5, 31, 42).

Because *Bartonella* species frequently induce persistent intravascular infections, it has been difficult to attribute chronic disease causation to infection in humans and companion animals; much of this difficulty may be related to the few and often very subtle clinical abnormalities that are reported by a patient or observed in a sick animal. Confirming disease causation is especially difficult in retrospective or prospective animal studies in which *Bartonella* bacteremia can be detected in overtly healthy, natural reservoir hosts—a paradigm in opposition to Koch's postulates for disease causation (12, 23). Nevertheless, an increasingly diverse spectrum of *Bartonella*-associated infections have been recognized in people and in dogs due to the development of new approaches to improving serologic and molecular diagnostic testing methods, which prove to be, in most instances, more sensitive than conventional culture methods for the isolation of *Bartonella* species (13, 15, 26, 30, 33, 38, 41, 46, 49). Primary isolation of *Bartonella* species following lysis centrifugation, or freezing of a blood sample, followed by application to a blood agar plate, is the most widely used method for the microbiological diagnosis of bartonellosis. Isolation of *Bartonella* species on a blood agar plate generally requires a prolonged incubation period (an average of 21 days) and is rarely successful, unless the patient or animal is infected with a retrovirus or is receiving immunosuppressive drug therapy, or unless the animal is a reservoir host for the given *Bartonella* species. To date, alternative methods of isolation have not proven to be of significant diagnostic utility, and no suitable liquid medium that will support the growth of all or most medically important *Bartonella* species has been described. Previous reports have described the growth of only one or a few *Bartonella* species, or isolation of *Bartonella* species only from experimentally infected animals (13, 18, 33).

In this report, we describe a novel liquid culture medium that will support the growth of at least seven *Bartonella* species. This medium will also support cocultures of different *Bartonella* species and may also facilitate the primary isolation of *B. henselae* from the blood and aqueous fluid of naturally infected cats.

MATERIALS AND METHODS

Bacterial strains, growth conditions, and chemicals. *Bartonella clarridgeiae* (ATCC 700095), *B. doshiae* (ATCC 700133), *B. elizabethae* (ATCC 49927), *B. grahamii* (ATCC 700132), *B. henselae* Houston-1 (ATCC 49882), *B. quintana* Fuller (ATCC VR-358), and *B. vinsonii* subsp. *berkhoffii* (ATCC 51672) were used for medium development and characterization. Liquid and solid cultures of *Bartonella* species were performed at 35°C in a 5% CO_2, water-saturated atmosphere. Liquid cultures were maintained with a constant shaking motion for 7 to 12 days. CFU counts in liquid cultures were determined at 24-h intervals after plating of 100-μl aliquots onto commercialized blood agar plates. Blood agar plates were then incubated at 35°C in a 5% CO_2, water-saturated atmosphere for 7 days before CFU enumeration. All chemicals and reagents were purchased from Sigma Chemicals (St. Louis, MO) unless stated otherwise.

Growth medium. The liquid growth medium described in this work (referred to below as *Bartonella-Alphaproteobacteria* growth medium [BAPGM]) was formulated on the basis of the biochemical composition of the insect growth medium DS2 from Mediatech (Herndon, VA). BAPGM was formulated to create an efficient growth medium for all of the *Bartonella* species described above. BAPGM was prepared by supplementing 900 ml of DS2 medium with 0.1 mg of NAD, 1.25 mg of NADP, 2 mg of ATP, 2 mg of sodium pyruvate, and 2 g of yeast extract. Amino acid supplementation was accomplished by adding 63.2 mg of L-arginine · HCl, 15.6 mg of L-cystine · HCl, 20.95 mg of L-histidine, 26.25 mg

* Corresponding author. Mailing address: Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606. Phone: (919) 513-8277. Fax: (919) 513-6336. E-mail: ed_breitschwerdt@ncsu.edu.

each of L-isoleucine and L-leucine, 36.25 mg of L-lysine, 7.5 mg of L-methionine, 16.25 mg of L-phenylalanine, 23.8 mg of L-threonine, 5 mg of L-tryptophan, 21.6 mg of L-tyrosine · 2Na · $2H_2O$, and 23.4 mg of L-valine. The pH of BAPGM was adjusted to 7.4 by addition of 50 ml of 0.1 M phosphate buffer, and BAPGM was subsequently sterilized by filtration through a 0.2-μm-pore-size filter (Corning, Corning, NY). After filtration, BAPGM was supplemented with 50 ml of defibrinated sheep blood (to a final concentration of 5%, vol/vol).

Growth experiments: single and multiple *Bartonella* species. In order to establish the growth-promoting characteristics of the medium, single as well as polymicrobial (two different species) *Bartonella* species were inoculated into BAPGM, after which the cultures were maintained at 35°C in a 5% CO_2, water-saturated atmosphere. Colonies of single *Bartonella* species were swabbed from the surface of 5- to 7-day-old blood agar plate subcultures and were resuspended in sucrose-phosphate-glutamate (SPG) buffer. An SPG suspension aliquot of 100 μl of *B. henselae* or *B. quintana* (for quantitative growth characterization), or of *B. clarridgeiae*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* (*berkhoffii*), or *B. doshiae* (for qualitative growth characterization) was inoculated into individual flasks containing 10 ml of BAPGM and cultured at 35°C. The growth of each liquid culture group was monitored for 7 to 12 days by inoculation of 100 μl onto blood agar plates every 24 h for colony quantification and by conventional PCR.

Coculture experiments were conducted by inoculating approximately 2×10^4 to 3×10^4 CFU (inoculum was subsequently determined by blood agar plate colony counts) of *B. henselae* and *B. quintana*, *B. henselae* and *B. vinsonii* (*berkhoffii*), or *B. quintana* and *B. vinsonii* (*berkhoffii*) into 10 ml of BAPGM. The growth of each liquid coculture was monitored by PCR testing at 3, 5, and 7 days following inoculation.

Naturally infected cat samples. Blood and/or aqueous fluid was submitted for diagnostic evaluation from two different cats (cat A and cat B). A 300-μl aliquot of EDTA-anticoagulated blood was submitted from cat A, which had a <1:16 antibody titer to *B. henselae* by indirect immunofluorescent antibody (IFA) testing conducted in our laboratory. Cat A was tested because the cat resides in the same household as a dog that was positive by real-time PCR for *B. henselae* DNA and had consistently elevated *B. henselae* IFA titers (1:64 and 1:128) over a 6-month period. Additionally, 300 μl of EDTA-blood and 100 μl of aqueous fluid from cat B were submitted because of severe uveitis; cat B had a *B. henselae* antibody titer of 1:256, and *B. henselae* DNA was detected in both the blood and aqueous fluid by conventional and real-time PCR. An aliquot (150 μl) of each of the two EDTA-blood samples and 300 μl of a 1:5 dilution in SPG buffer of the aqueous fluid sample from cat B were inoculated into 5 ml of BAPGM and cultured as described above for 7 days. Growth and colony formation in liquid cultures were determined by plating 100-μl aliquots onto commercialized blood agar plates and incubating at 35°C in a 5% CO_2, water-saturated atmosphere for 7 days. Colonies observed on the blood agar plates were subsequently isolated for characterization by species-specific conventional PCR. The remaining150-μl aliquots of each of the two EDTA-blood samples and 200 μl of the 1:5 dilution of the cat B aqueous fluid sample were inoculated onto commercialized blood agar plates and incubated at 35°C in a 5% CO_2, water-saturated atmosphere for 5 weeks.

DNA extraction and PCR screening of bacterial cultures. Screening of each *Bartonella* species in liquid and solid media from single-organism cultures, cocultures, and isolates of the two naturally infected cats was performed by conventional PCR. DNA was prepared from 200 μl of each liquid culture, or from a resuspension (in Tris-EDTA buffer) of several colonies from the blood agar plate isolates using the QIAamp DNA minikit (QIAGEN Inc., Valencia, CA). After extraction, DNA concentration and purity were measured using an absorbance ratio between 260 and 280 nm.

Conventional PCR analysis. PCR species screening was performed using primers designed to amplify a consensus sequence in the *Bartonella* intergenic transcribed sequence (ITS) region. The amplicon size of this region is species dependent, with sizes ranging from 453 bp for *B. bovis* to 717 bp for *B. elizabethae* (34). Oligonucleotides 321s (5′ AGA TGA TGA TCC CAA GCC TTC TGG 3′) and 983as (5′ TGT TCT YAC AAC AAT GAT GAT G 3′) were used as forward and reverse primers, respectively. Amplification of the ITS region was performed in a reaction mixture with a 25-μl final volume containing 16.5 μl of molecular-grade water (Epicentre), 0.5 μl of 10 mM deoxynucleoside triphosphate mixture, 2.5 μl of 10× PCR buffer, 2.5 μl of 25 mM $MgCl_2$, and 0.7 U of Amplitaq Gold DNA polymerase. All reagents were purchased from Perkin-Elmer Applied Biosystems (Foster City, CA) unless otherwise stated. The reaction mixture was completed by adding 0.25 μl of 30 μM each forward and reverse primer (IDT DNA Technology) and 2 μl of DNA from each *Bartonella* species tested. PCR-negative controls consisted of 2 μl of distilled H_2O (when isolates from plates were tested) or 2 μl of DNA extracted from uninoculated BAPGM (when BAPGM cultures were tested). Conventional PCR conditions were as follows: a single hot-start cycle at 95°C for 5 min, followed by 45 cycles of denaturing at 94°C for 45 seconds, annealing at 54°C for 45 seconds, and extension at 72°C for 45 seconds. Amplification was completed by an additional cycle at 72°C for 10 min, and products were analyzed by 2% agarose gel electrophoresis and detection using ethidium bromide under UV light.

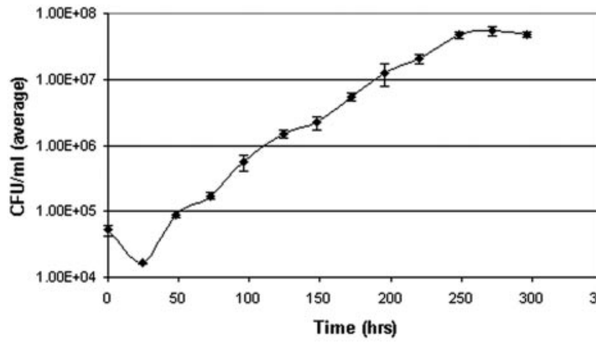

FIG. 1. Growth curve of *B. henselae* in BAPGM. Viable cell counts (reported as CFU/ml) in liquid BAPGM were determined at 24-h intervals after plating of individual culture aliquots onto commercial blood agar plates. Colony counts were performed after a 7-day incubation period on blood agar plates that were maintained at 35°C and 5% CO_2 in a water-saturated atmosphere.

Real-time PCR analysis. The Scorpion 321 fluorescent probe (5′ FAM-CCG CGT TTT TCA AAG CCC ACG CGG-QUE-HEG-AGA TGA TGA TCC CAA GCC TTC TGG 3′) and primer 425as (5′ GGA TRA AYY RGW AAA CCT TYM YCG G 3′) were used for PCR genus screening of the *Bartonella* ITS region. *Bartonella henselae* species identification was performed by real-time PCR using a Taqman fluorescent probe (Cy5-CCA CCG TGG GCT TTG AAA AAC GCT-DBHQ3) and oligonucleotides 321s (5′ AGA TGA TGA TCC CAA GCC TTC TGG CG 3′) and 421 as (5′ GGA TRA AYY RGW AAA CCT TYM YCG G 3′) as forward and reverse primers (IDT DNA Technology), respectively. Real-time reactions were performed using a SmartCycler II system (Cepheid, Sunnyvale, CA) with reaction mixtures (final volume, 25 μl) containing 14.5 μl of molecular-grade water (Epicentre), 5 μl Takara PCR Master Mix, and 2 μl of 25 mM $MgCl_2$. All reagents were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise stated. As above, 2 μl of DNA from each sample was used for real-time PCR analysis. Reactions were completed by adding either 1 μl of 10 μM Scorpion 321 fluorescent probe and 0.25 μl of 30 μM primer 425as (for genus diagnostics) or 1 μl of 10 μM Taqman probe and 0.25 μl of 30 μM each primer, 321s and 425as (for species identification). Real-time PCR conditions were as follows: a single hot-start cycle at 95°C for 30 s, followed by 45 cycles of denaturing at 94°C for 10 s, 6 s of annealing at 58°C (for *Bartonella* genus diagnostics) or 54°C (for *B. henselae* diagnostics), and a final extension at 72°C for 10 s. Positive amplicons were detected by fluorescence reading at the appropriate wavelength.

RESULTS

Growth of *Bartonella* cultures. The changes in CFU following culture of *B. henselae* and *B. quintana* in BAPGM for 7 days resulted in growth curves with the three typical phases of bacterial cell growth: the lag phase, the logarithmic-growth phase, and the stationary, or death, phase. The growth of *B. henselae* (Fig. 1) in BAPGM was characterized by a 72-h lag phase, with initial colony counts of approximately 5×10^4 CFU/ml (standard deviation [SD], 1×10^4), followed by a reduction to 1.6×10^4 CFU/ml (SD, 5×10^3) 24 h later. After a 96-h incubation in BAPGM, logarithmic growth was observed, with a maximum CFU/ml of 5.4×10^7 (SD, 8×10^6) at 272 h. The growth rate under these culture conditions revealed a 24-h division time for *B. henselae*. After *B. henselae* reached the maximum growth at

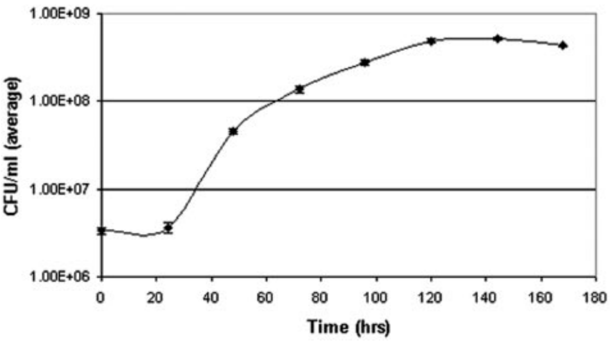

FIG. 2. Growth curve of *B. quintana* in BAPGM. Viable cell counts (reported as CFU/ml) in liquid BAPGM were determined at 24-h intervals after plating of individual culture aliquots onto commercial blood agar plates. Colony counts were performed after a 7-day incubation period on blood agar plates that were maintained at 35°C and 5% CO_2 in a water-saturated atmosphere.

272 h, a rapid decrease in CFU/ml was observed, with no apparent steady-state stationary phase.

Pure cultures of *B. quintana* had a lag phase of 24 to 48 h (Fig. 2), with an initial CFU/ml of approximately 3.3×10^6 (SD, 3×10^5). This phase was followed by a logarithmic-growth phase that reached a maximum of 5.2×10^8 CFU/ml (SD, 2×10^7) at 120 h postinoculation. The growth rate under these culture conditions revealed a 24-h division time. As with *B. henselae*, a rapid decrease in CFU/ml was observed for *B. quintana*, with no plateau observed during stationary phase.

Likewise, the maximum onset of bacterial growth in BAPGM for *B. clarridgeiae*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* (*berkhoffii*), and *B. doshiae* cultures (as observed by blood agar plating) averaged 7 to 10 days postinoculation (results not shown). As with *B. henselae* and *B. quintana*, PCR screening for the *Bartonella* ITS region on all BAPGM cultures at 7 days after inoculation resulted in bands of the appropriate size (i.e., matching each cultured species) by gel electrophoresis (Fig. 3).

Coculture of *Bartonella* species. In all coculture experiments, BAPGM supported the growth of each species, and their respective identities were confirmed by conventional PCR screening of the ITS region. Initial PCR screening of 24-h coculture samples showed slight bands corresponding to each *Bartonella* species' ITS amplicon size (results not shown). PCR results obtained from coculture samples at 5 days postinoculation (Fig. 3) showed stronger PCR bands matching each species inoculated into the respective culture: bands at 648 bp and at 564 bp for coculture of *B. henselae* and *B. quintana*, respectively (Fig. 3, lane 13), bands at 648 bp and at 706 bp for coculture of *B. henselae* and *B. vinsonii* (*berkhoffii*), respectively (Fig. 3, lane 14), and bands at 706 bp and at 564 bp for coculture of *B. vinsonii* (*berkhoffii*) and *B. quintana*, respectively (Fig. 3, lane 15). Band intensities for each species inoculated as cocultures at 5 days postinoculation matched the bands for each single species that was inoculated and examined under the same culture conditions (Fig. 3, lanes 10 to 12). However, at 7 days postinoculation, the *B. vinsonii* (*berkhoffii*) band disappeared from both the *B. henselae–B. vinsonii* (*berkhoffii*) and *B. quintana–B. vinsonii* (*berkhoffii*) cocultures, but not from the liquid cultures containing *B. vinsonii* (*berkhoffii*) as a single culture species (results not shown). No such effect was observed in the *B. henselae* and *B. quintana* coculture, in which the 648-bp and 564-bp bands remained clearly visible for as long as 10 days.

Isolation of *B. henselae* from naturally infected cats. No growth was observed from the two blood samples or from the aqueous fluid sample after direct inoculation onto a blood agar plate and visual monitoring of the cultures for 5 weeks. Culture of the blood sample from cat A and the aqueous fluid from cat B in BAPGM for 7 days, followed by subculturing onto a blood agar plate, produced colonies that were clearly visible on the blood agar plate after 7 days. Three colonies were obtained from the cat A blood sample, and several colonies were obtained from the aqueous fluid sample (cat B). Each colony was subcultured on blood agar plates for species identification. Samples obtained directly from BAPGM after 3 and 7 days, as well as from each blood agar plate isolate, were screened by conventional PCR. For all the samples, PCR followed by gel electrophoresis resulted in a 648-bp single-band amplicon that corresponds to the ITS region amplicon of *B. henselae*. Further, this finding was confirmed by real-time PCR using a *B. henselae*-specific Taqman fluorescent probe. Although no colony formation was observed following subculture of the cat B blood sample, real-time PCR analyses using DNA extracted from whole blood and from BAPGM blood culture were both positive by use of probes specific for *Bartonella* genera and for *B. henselae*. It is interesting that *B. henselae* in the blood and

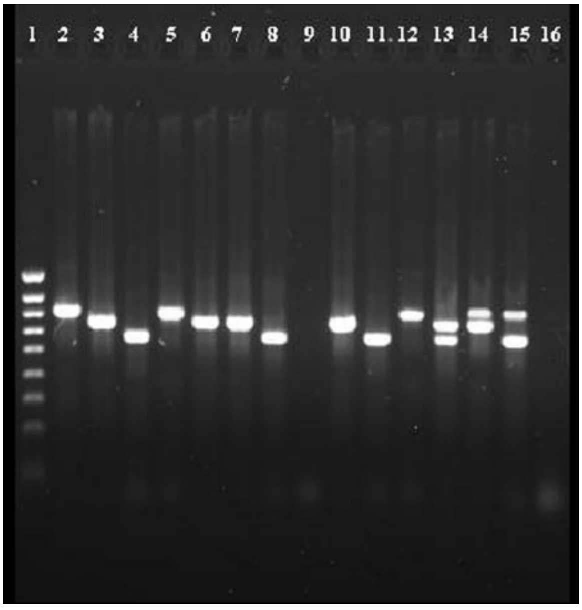

FIG. 3. Two percent agarose gel electrophoresis of an intergenic spacer region PCR amplification (primers 321s and 983as) of *Bartonella* cultures in BAPGM (lanes 2 to 9, at 7 days postinoculation; lanes 10 to 16, 5 days postinoculation). Lane 1, 1-kbp DNA ladder; lane 2, *B. elizabethae* (717 bp); lane 3, *B. grahamii* (658 bp); lane 4, *B. quintana* Fuller (564 bp); lanes 5 and 12, *B. vinsonii* (*berkhoffii*) (706 bp); lane 6, *B. henselae* Houston-1 (648 bp); lane 7, *B. clarridgeiae* (636 bp); lane 8, *B. doshiae* (550 bp); lanes 9 and 16, BAPGM as a negative control; lane 10, *B. henselae*; lane 11, *B. quintana*; lane 13, *B. henselae* and *B. quintana* coculture; lane 14, *B. henselae* and *B. vinsonii* (*berkhoffii*) coculture; lane 15, *B. quintana* and *B. vinsonii* (*berkhoffii*) coculture.

aqueous fluid samples from cat B showed a much lower DNA copy number in the blood and in the blood-BAPGM culture by real-time PCR than in the aqueous fluid and the aqueous fluid-BAPGM culture (results not shown). Successful isolation of *B. henselae* from the aqueous fluid from cat B may have resulted due to the larger number of bacteria present in this sample, compared with the number of organisms present in blood.

DISCUSSION

As is true for many fastidious pathogens, difficulties associated with *Bartonella* isolation have compromised efforts to define the role of these organisms in disease causation. Isolation of the infecting bacteria aids in the evaluation of more sensitive and improved diagnostic assays and advances the understanding of the diversity, adaptation, and epidemiology of this genus (33). It is our opinion that chronic infection with *Bartonella* species can contribute to very subtle clinical abnormalities or vague symptoms in companion animals or human patients, respectively. Despite advances in PCR, improved culture methods are needed for the isolation of *Bartonella* species. Culture media used for *Bartonella* species isolation in clinical laboratories have questionable sensitivity, and many laboratories continue to rely on standard blood agar plate cultures (5% rabbit blood heart infusion-Trypticase agar or chocolate agar) (10, 13, 33).

Several laboratories have participated in research efforts with the goal of creating an isolation medium that will enhance the growth and maintenance of *Bartonella* species. Despite these efforts, isolation of *Bartonella* species from nonimmunocompromised individuals remains a highly insensitive diagnostic method. This finding suggests that additional optimization of the biochemical constituents of a liquid medium is required in order to obtain isolates from patients. To date, no liquid- or solid-phase medium has proven to be reliable for the isolation of single or multiple *Bartonella* species from naturally infected animals or humans (3, 13). Although some blood-free media have shown good potential for the growth of *B. henselae* or *B. quintana* (13, 49), supplementation with blood, erythrocyte membranes, or an erythrocyte membrane component seems to be required for initial growth and to achieve the full growth-promoting effect of the culture medium (33, 46). In addition, the use of hemin as a blood or erythrocyte substitute represents a real challenge, which could compromise the use of these media when the infecting *Bartonella* species is not known or when polymicrobial *Bartonella* infection is present in the patient. Research has shown that optimal hemin concentrations differ among *Bartonella* species (46). *Bartonella quintana* required a hemin concentration of 40 μg/ml for growth, while *B. henselae* required approximately 250 μg/ml of hemin for ideal growth. In fact, hemin concentrations in excess of these established for optimal growth became toxic to each *Bartonella* species tested, resulting in decreased growth (i.e., ideal hemin concentrations for the growth of *B. henselae* are toxic to *B. quintana*) (46). It should be noted that only *B. henselae* (from culture and clinical samples) and *B. quintana* (from culture samples) have been evaluated in order to establish the potential isolation and growth support/enhancement role of blood-free media (13, 46, 49). Also, the development of optimal culture techniques and improvements in isolation media may have been hampered somewhat by the fact that only a few (primarily *B. henselae* and *B. quintana*) of the 17 *Bartonella* species described to date have been biochemically characterized.

In this report, we describe the development of a novel liquid isolation medium, which is based on a formulation that promotes the growth of insect cells in culture. This medium supports the isolation and growth of at least seven *Bartonella* species and may facilitate the isolation of two or more *Bartonella* species from the same patient. Coinfection with more than one *Bartonella* species, as indicated by serology and/or PCR, has frequently been reported in dogs and cats (19, 20, 36, 47) and has been suggested to occur in rodents (32, 50). It is our opinion that the occurrence of coinfection with multiple *Bartonella* species is likely underestimated in both animals and humans due to the limitations associated with the culture and isolation of the infecting agent and the limitations associated with other diagnostic methodologies currently in use (e.g., PCR and serology). It is not clear if the disappearance of the 706-bp band of *B. vinsonii* (*berkhoffii*) during coculture with *B. henselae* or *B. quintana* was a consequence of the death of this species as a result of competition, an effect of the production of a toxic metabolite(s) by *B. henselae* and *B. quintana*, or a deleterious effect of phage induction (2, 4, 13, 35). If these in vitro data are applicable to competition among *Bartonella* species within animals or human beings, this would suggest that one *Bartonella* species could substantially suppress another *Bartonella* species, making detection by culture or PCR very difficult. In the present study, BAPGM supported the primary isolation of *B. henselae* from two clinical samples (blood and aqueous fluid) in half the time generally required when a conventional isolation medium (e.g., a sheep blood agar plate or chocolate agar plate) is used. Isolates were not obtained from these two clinical samples by direct inoculation onto a blood agar plate, consistent with the limited sensitivity of the conventional isolation approach for detection of *B. henselae* and other *Bartonella* species. Recently, we have used the isolation approach described in this study to obtain *Bartonella* species isolates from dogs and humans with chronic illnesses.

The results of this study suggest that the use of BAPGM for the detection and isolation of *Bartonella* species may provide an improved or alternative method to isolate these fastidious microorganisms from patient samples. In the future, BAPGM may represent an important culture option for the clinical microbiology laboratory. In an effort to optimize the utility of this medium, we are currently evaluating the effectiveness of different amino acid combinations in BAPGM. Amino acid supplementation appears to be one of the key components required to improve and enhance *Bartonella* species growth (13). In addition, we are further evaluating the diagnostic utility of BAPGM for the improved isolation of *Bartonella* species from sick animals and humans.

REFERENCES

1. **Anderson, B., and M. A. Neuman.** 1997. *Bartonella* spp. as emerging human pathogens. Clin. Microbiol. Rev. **10:**203–219.
2. **Anderson, B. E., C. Goldsmith, A. Johnson, I. Padmalayam, and B. Baumstark.** 1994. Bacteriophage-like particles of *Rochalimaea henselae.* Mol. Microbiol. **13:**67–73.
3. **Avidor, B., M. Graidy, G. Efrat, C. Leibowitz, G. Shapira, A. Schattner, O. Zimhony, and M. Giladi.** 2004. *Bartonella koehlera*, a new cat-associated agent of culture-negative human endocarditis. J. Clin. Microbiol. **42:**3462–3468.

4. **Barksdale, L., and S. B. Arden.** 1974. Persisting bacteriophage infections, lysogeny, and phage conversions. Rev. Microbiol. **28:**265–299.
5. **Bergmans, A. M. C., J. F. P. Schellekens, J. D. A. Van Embden, and L. M. Schouls.** 1996. Predominance of two *Bartonella* variants among cat scratch disease patients in The Netherlands. J. Clin. Microbiol. **34:**254–260.
6. **Birtles, R. J., J. Canales, P. Ventosilla, E. Alvarez, H. Guerra, A. Llanos-Cuentas, D. Raoult, N. Doshi, and T. G. Harrison.** 1999. Survey of *Bartonella* species infecting intradomicillary animals in the Huayllacallan Valley, Ancash, Peru, a region endemic for human bartonellosis. Am. J. Trop. Med. Hyg. **60:**799–805.
7. **Breitschwerdt, E. B., C. E. Atkins, T. T. Brown, D. L. Kordick, and P. S. Snyder.** 1999. *Bartonella vinsonii* subsp. *berkhoffi* and related members of the alpha subdivision of the *Proteobacteria* in dogs with cardiac arrhythmias, endocarditis, or myocarditis. J. Clin. Microbiol. **37:**3618–3626.
8. **Breitschwerdt, E. B., and D. L. Kordick.** 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. Clin. Microbiol. Rev. **13:**428–438.
9. **Breitschwerdt, E. B., D. L. Kordick, and D. E. Malarkey.** 1995. Endocarditis in a dog due to infection with a novel *Bartonella* subspecies. J. Clin. Microbiol. **33:**154–160.
10. **Brenner, S. A., J. A. Rooney, P. Manzewitsch, and R. L. Regnery.** 1997. Isolation of *Bartonella* (*Rochalimaea*) *henselae*: effects of methods of blood collection and handling. J. Clin. Microbiol. **35:**544–547.
11. **Brouqui, P., B. La Scola, V. Roux, and D. Raoult.** 1999. Chronic *Bartonella quintana* bacteremia in homeless patients. N. Engl. J. Med. **340:**184–189.
12. **Chang, C. C., B. B. Chomel, R. W. Kasten, R. Heller, K. M. Kocan, H. Ueno, K. Yamamoto, V. C. Bleich, B. M. Pierce, B. J. Gonzales, P. K. Swift, W. M. Boyce, S. S. Jang, H. J. Boulouis, and Y. Piémont.** 2000. *Bartonella* spp. isolated from wild and domestic ruminants in North America. Emerg. Infect. Dis. **6:**306–311.
13. **Chenoweth, M. R., G. A. Somerville, D. C. Krause, K. L. O'Reilly, and F. C. Gherardini.** 2004. Growth characteristics of *Bartonella henselae* in a novel liquid medium: primary isolation, growth-phase-dependent phage induction, and metabolic studies. Appl. Environ. Microbiol. **70:**656–663.
14. **Clarridge, J. E., III, T. J. Raich, D. Pirwani, B. Simon, L. Tsai, M. C. Rodriguez-Barradas, R. L. Regnery, A. Zollo, D. C. Jones, and C. Rambo.** 1995. Strategy to detect and identify *Bartonella* species in routine clinical laboratory yields *Bartonella henselae* from human immunodeficiency virus-positive patient and unique *Bartonella* strain from his cat. J. Clin. Microbiol. **33:**2107–2113.
15. **Cunningham, E. T., and J. E. Koehler.** 2000. Ocular bartonellosis. Am. J. Ophthalmol. **130:**340–349.
16. **Daly, J. S., M. G. Worthington, D. J. Brenner, C. W. Moss, D. G. Hollis, and R. S. Weyant.** 1993. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J. Clin. Microbiol. **31:**872–881.
17. **Drancourt, M., R. Birtles, G. Chaumentin, F. Vandenesch, J. Etienne, and D. Raoult.** 1996. New serotype of *Bartonella henselae* in endocarditis and cat-scratch disease. Lancet **347:**441–443.
18. **Fournier, P. E., J. Robson, Z. Zeaiter, R. McDougall, S. Byrne, and D. Raoult.** 2002. Improved culture from lymph nodes of patients with cat scratch disease and genotypic characterization of *Bartonella henselae* isolates in Australia. J. Clin. Microbiol. **40:**3620–3624.
19. **Gurfield, A. N., H. J. Boulouis, B. B. Chomel, R. Heller, R. W. Kasten, K. Yamamoto, and Y. Piemont.** 1997. Coinfection with *Bartonella clarridgeiae* and *Bartonella henselae* and with different *Bartonella henselae* strains in domestic cats. J. Clin. Microbiol. **35:**2120–2123.
20. **Gurfield, A. N., H. J. Boulouis, B. B. Chomel, R. W. Kasten, R. Heller, C. Bouillin, C. Gandoin, D. Thibault, C. C. Chang, F. Barrat, and Y. Piemont.** 2001. Epidemiology of *Bartonella* infection in domestic cats in France. Vet. Microbiol. **80:**185–198.
21. **Hadfield, T. L., R. Warren, M. Kass, E. Brun, and C. Levy.** 1993. Endocarditis caused by *Rochalimaea henselae.* Hum. Pathol. **24:**1140–1141.
22. **Houpikian, P., and D. Raoult.** 2001. 16S/23S rRNA intergenic spacer regions for phylogenetic analysis, identification, and subtyping of *Bartonella* species. J. Clin. Microbiol. **39:**2768–2778.
23. **Jacomo, V., P. J. Kelly, and D. Raoult.** 2002. Natural history of *Bartonella* infections (an exception to Koch's postulate). Clin. Diagn. Lab. Immunol. **9:** 8–18.
24. **Jensen, W. A., M. Z. Fall, J. Rooney, D. L. Kordick, and E. B. Breitschwerdt.** 2000. Rapid identification and differentiation of *Bartonella* species using a single-step PCR assay. J. Clin. Microbiol. **38:**1717–1722.
25. **Joblet, C., V. Roux, M. Drancourt, J. Gouvernet, and D. Raoult.** 1995. Identification of *Bartonella* (*Rochalimaea*) species among fastidious gram-negative bacteria on the basis of the partial sequence of the citrate synthase gene. J. Clin. Microbiol. **33:**1879–1883.
26. **Johnson, G., M. Ayers, S. C. McClure, S. E. Richardson, and R. Tellier.** 2003. Detection and identification of *Bartonella* species pathogenic for humans by PCR amplification targeting the riboflavin synthase gene (*ribC*). J. Clin. Microbiol. **41:**1069–1072.
27. **Karem, K. L., C. D. Paddock, and R. L. Regnery.** 2000. *Bartonella henselae*, *B. quintana*, and *B. bacilliformis*: historical pathogens of emerging significance. Microbes Infect. **2:**1193–1205.
28. **Kerkhoff, F. T., A. M. Bergmans, A. van Der Zee, and A. Rothova.** 1999. Demonstration of *Bartonella grahamii* DNA in ocular fluids of a patient with neuroretinitis. J. Clin. Microbiol. **37:**4034–4038.
29. **Koehler, J. E., M. A. Sanchez, S. Tye, C. S. Garrido-Rowland, F. M. Chen, T. Maurer, J. L. Cooper, J. G. Olson, A. L. Reingold, W. K. Hadley, R. R. Regnery, and J. W. Tappero** 2003. Prevalence of *Bartonella* infection among human immunodeficiency virus-infected patients with fever. Clin. Infect. Dis. **37:**559–566.
30. **Koehler, J. E., F. D. Quinn, T. G. Berger, P. E. LeBoit, and J. W. Tappero.** 1992. Isolation of *Rochalimaea* species from cutaneous and osseous lesions of bacillary angiomatosis. N. Engl. J. Med. **327:**1625–1631.
31. **Kordick, D. L., E. J. Hilyard, T. L. Hadfield, K. H. Wilson, A. G. Steigerwalt, D. J. Brenner, and E. B. Breitschwerdt.** 1997. *Bartonella clarridgeiae*, a newly recognized zoonotic pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch disease). J. Clin. Microbiol. **35:**1813–1818.
32. **Kosoy, M. Y., R. L. Regnery, T. Tzianabos, E. L. Marston, D. C. Jones, D. Green, G. O. Maupin, J. G. Olson, and J. E. Childs.** 1997. Distribution, diversity, and host specificity of *Bartonella* in rodents from the Southeastern United States. Am. J. Trop. Med. Hyg. **57:**578–588.
33. **LaScola, B., and D. Raoult.** 1999. Culture of *Bartonella quintana* and *Bartonella henselae* from human samples: a 5-year experience (1993–1998). J. Clin. Microbiol. **37:**1899–1905.
34. **Maggi, R. G., and E. B. Breitschwerdt.** 2005. Potential limitations of the 16S–23S rRNA intergenic region for molecular detection of *Bartonella* species. J. Clin. Microbiol. **43:**1171–1176.
35. **Maggi, R. G., and E. B. Breitschwerdt.** 2004. Bacteriophage in *Bartonella vinsonii berkhoffii* and characterization of its Pap 31 gene. The 4th International Conference on *Bartonella* as Emerging Pathogens, Uppsala, Sweden.
36. **Maruyama, S., T. Sakai, Y. Morita, S. Tanaka, H. Kabeya, S. Boonmar, A. Poapolathep, T. Chalarmchaikit, C. C. Chang, R. W. Kasten, B. B. Chomel, and Y. Katsube.** 2001. Prevalence of *Bartonella* species and 16S rRNA gene types of *Bartonella henselae* from domestic cats in Thailand. Am. J. Trop. Med. Hyg. **65:**783–787.
37. **Maurin, M., R. Birtles, and D. Raoult.** 1997. Current knowledge of *Bartonella* species. Eur. J. Clin. Microbiol. Infect. Dis. **16:**487–506.
38. **Pitulle, C., C. Strehse, J. W. Brown, and E. B. Breitschwerdt.** 2002. Investigation of the phylogenetic relationships within the genus *Bartonella* based on comparative sequence analysis of the *rnpB* gene, 16S rDNA, and 23S rDNA. Int. J. Syst. Evol. Microbiol. **52:**2075–2080.
39. **Raoult, D.** 1995. *Bartonella* (*Rochalimaea*) *quintana* endocarditis in three homeless men. N. Engl. J. Med. **332:**419–423.
40. **Raoult, D., M. Drancourt, A. Carta, and J. A. Gastaut.** 1994. *Bartonella* (*Rochalimaea*) *quintana* isolation in a patient with chronic adenopathy, lymphopenia, and a cat. Lancet **343:**977.
41. **Raoult, D., G. Greub, P. E. Fournier, and Z. Zeaiter.** 2003. Diagnosis of *Bartonella* endocarditis by real-time nested PCR assay using serum. J. Clin. Microbiol. **41:**919–925.
42. **Regnery, R. L., B. E. Anderson, J. E. Clarridge III, M. Rodriguez-Barradas, D. C. Jones, and J. H. Carr.** 1992. Characterization of a novel *Rochalimaea* species, *R. henselae* sp. nov., isolated from blood of a febrile human immunodeficiency virus-positive patient. J. Clin. Microbiol. **30:**265–274.
43. **Relman, D. A.** 1995. Has trench fever returned? N. Engl. J. Med. **332:**463–464.
44. **Schattner, A., O. Zimhony, B. Avidor, and M. Giladi.** 2003. Asking the right question. Lancet **361:**1786.
45. **Schwartzman, W.** 1996. *Bartonella* (*Rochalimaea*) infections: beyond cat scratch. Annu. Rev. Med. **47:**355–364.
46. **Schwartzman, W. A., C. A. Nesbit, and E. J. Baron.** 1993. Development and evaluation of a blood-free medium for determining growth curves and optimizing growth of *Rochalimaea henselae.* J. Clin. Microbiol. **31:**1882–1885.
47. **Solano-Gallego, L., J. Bradley, B. Hegarty, B. Sigmon, and E. Breitschwerdt.** 2004. *Bartonella henselae* IgG antibodies are prevalent in dogs from south-eastern USA. Vet. Res. **35:**585–595.
48. **Welch, D. F., D. A. Pickett., L. N. Slater, A. G. Steigerwalt, and D. J. Brenner.** 1992. *Rochalimaea henselae* sp. nov., a cause of septicemia, bacillary angiomatosis, and parenchymal bacillary peliosis. J. Clin. Microbiol. **30:** 275–280.
49. **Wong, M. T., M. J. Dolan, C. P. Lattuada, R. L. Regnery, M. L. Garcia, and E. C. Mokulis.** 1995. Neuroretinitis, aseptic meningitis, and lymphadenitis associated with *Bartonella henselae* infection in immunocompetent patients and patients infected with human immunodeficiency virus type I. Clin. Infect. Dis. **21:**352–360.
50. **Ying, B., M. Y. Kosoy, G. O. Maupin, K. R. Tsuchiya, and K. L. Gage.** 2002. Genetic and ecologic characteristics of *Bartonella* communities in rodents in southern China. Am. J. Trop. Med. Hyg. **66:**622–627.